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# **DIMINISHED FOOD RESOURCES ARE ASSOCIATED WITH DELAYED REPRODUCTION OR INCREASED POST-REPRODUCTIVE MORTALITY IN BROOD-BEARING TERRESTRIAL ISOPODS *ARMADILLIDIUM VULGARE* LATREILLE<sup>1</sup>**

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**ABSTRACT:** Female terrestrial isopods (Crustacea: Oniscidea) carry eggs and early instars in a ventral brood pouch. We investigated reproductive expenditure of female *Armadillidium vulgare* Latreille under the condition of restricted food resources. Regardless of food availability, few cases of spontaneous termination of care were observed and most gravid females either successfully produced offspring or died while still bearing eggs. There were no differences in pre-hatching maternal mortality between food-restricted and non-restricted groups, but females exhibited significantly higher post-reproductive mortality when food availability was heavily reduced after oogenesis. This did not occur when food was restricted prior to oogenesis, but in this case females delayed the onset of reproduction. An association between mortality and past reproduction was further supported by high laboratory mortality, regardless of food availability, in non-gravid females field-captured late in the reproductive season. Maternal investment in *A. vulgare* thus appears to be energetically expensive. Despite the ability to terminate care, however, females continue to invest heavily in reproduction even when resources are scarce and the likelihood of mortality is high.

**KEY WORDS:** *Armadillidium vulgare*, Isopoda, diminished food resources, delayed reproduction, post-reproductive mortality, brood-rearing.

The evolutionary significance of arthropod parental care has been reviewed elsewhere (Tallamy 1984; Tallamy and Wood 1986; Kaitala and Mappes 1992), but studies of the phenomenon are generally limited to the Insecta where it has independently evolved in several taxa. Subsocial behavior, however, also evolved in the Crustacea and can be readily observed in the terrestrial Isopoda (e.g. Linsenmair 1987). Unlike the majority of subsocial insects, which generally brood eggs deposited on external surfaces, a female terrestrial isopod bears eggs and early-instar young (manca) in a ventral marsupium (a fluid-filled pouch formed by oostegites on the ventral pereon). This places a number of unique constraints upon the reproductive success of female terrestrial isopods, including physical demands on locomotion (Kight and Ozga 2001) and spatial limits on fecundity (Tomescu et al. 1992; Dangerfield and Telford 1995).

Because reproductive success is constrained by egg-bearing, terrestrial isopods may have evolved behavioral or physiological plasticity in the face of changing environmental conditions. To optimize reproductive success, female isopods should alter the magnitude of parental investment depending on the availability of resources such as food and favorable habitats. For example, Rush-ton and Hassall (1983) observed that female *Armadillidium vulgare* Latreille

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reared on different food sources exhibited differences in fecundity. Isopod fecundity can also be negatively affected by competition among individuals for limited resources (Hassall and Dangerfield 1997).

The present study examines the reproductive biology of female *A. vulgare* under limited food resources. We first investigate the spontaneous termination of care and patterns of mortality in food-restricted females during the first reproductive episode of the season (reproduction is seasonal in temperate climates (Souty-Grosset et al. 1998). Gravid females may respond to food restriction in three possible ways: an increase of parental investment in the face of diminished resources, reduction (including termination) of investment, or no change in patterns of investment at all.

We next examine post-reproductive responses to food stress in females captured at the end of the reproductive season. Three alternative hypotheses may again be considered. If the act of brood bearing places burdens on maternal expenditure only in the short-term, post-reproductive, food-restricted females should have lower mortality than reproductive food-restricted females. Alternatively, if brood bearing reduces long-term residual reproductive potential, post-reproductive, food-restricted females are expected to suffer similar or even greater mortality than gravid food-restricted females. Finally, brood bearing may have no effect on mortality and post-reproductive females should not differ from reproductive females.

Finally, we test the hypothesis that females exposed to food stress prior to the first reproductive episode will respond differently than females stressed only after oogenesis. If limited food resources constrain oogenesis and the onset of reproduction, pre-reproductive females should reduce or delay investment. There are two additional alternatives: food-restricted, pre-reproductive females could increase investment (although this seems unlikely) or exhibit unaltered patterns of reproductive allocation.

## METHODS I

### FOOD RESTRICTION DURING THE BROODING PHASE

*Armadillidium vulgare* Latreille were hand-collected twice during the reproductive season of 2001 in Essex County, New Jersey, USA. The first sample was taken during early May, whereas the second sample was obtained in late August. Animals were collected from the same location in both cases and therefore our samples are presumed to be from a single population. Collected animals were returned to the laboratory and maintained at 21°C and a 15L:9D light/dark photocycle in ventilated plastic enclosures containing moist cellulose sponge and carrots administered *ad libitum*.

Upon evidence of egg-bearing (determined by visual inspection), females in the May sample were isolated in individual *Drosophila* culture vials and divided into three treatment groups by matched triads according to estimated body size. Control females were provided unlimited access to carrots throughout the exper-

iment. The second group was moderately food restricted by a feeding cycle in which carrots were provided for four consecutive days followed by removal of food for two days. The third group was heavily food restricted by a feeding cycle in which carrots were provided for two consecutive days followed by removal of food for four days. Each treatment group contained 30 females and was monitored for 17 days. During this period females were examined daily for either the continued presence of eggs, the occurrence of spontaneous termination of care, or the death of the subject.

Females from the August sample did not reproduce in the laboratory. Specimens were isolated into individual *Drosophila* culture vials and divided into two treatment groups by matched pairs according to estimated body size. Control females were provided unlimited access to carrots while the second group was heavily food restricted as described. Each treatment group contained 30 females examined daily for mortality over a period of 17 days.

## RESULTS I

### FOOD RESTRICTION DURING THE BROODING PHASE

Data were analyzed following Gravetter and Wallnau (1988) using *Statistix* v.2.0 statistical software with  $\alpha = 0.05$ .

Few cases of spontaneous termination of care were observed, regardless of experimental treatment. Three control females, two moderately restricted and five heavily restricted females changed from an obvious gravid state to a non-gravid state over the course of the study ( $N = 90$ ,  $\chi^2 = 1.575$ ,  $DF = 2$ ,  $P = 0.4550$ ). In most cases the mechanism of termination was unknown, although we occasionally observed terminating females with eggs protruding from the marsupium and in some cases even feeding upon the eggs.

In the remaining reproductive females there were no differences among treatment groups in pre-hatching maternal mortality. Two control females, four moderately restricted and six heavily restricted females died without hatching young ( $N = 80$ ,  $\chi^2 = 2.820$ ,  $DF = 2$ ,  $P = 0.2441$ ). There was, however, significantly higher post-reproductive mortality in the heavily restricted treatment group (Fig. 1) during the observation period ( $N = 68$ ,  $\chi^2 = 15.67$ ,  $DF = 2$ ,  $P = 0.0004$ ). Fifteen heavily restricted females died shortly after the appearance of offspring, whereas this occurred in only 6 control and 7 moderately restricted females.

Mortality was also high in the presumably post-reproductive females captured in August. There were, however, no differences between the two late-season, treatment groups: 17 control females and 23 food-restricted females died during the study period ( $N = 60$ ,  $\chi^2 = 2.70$ ,  $DF = 1$ ,  $P = 0.1000$ ). While the late-season, food-restricted females did not differ from early-season reproductive food-restricted females in overall mortality ( $N = 60$ ,  $\chi^2 = 0.34$ ,  $DF = 1$ ,  $P = 0.5000$ ), overall mortality was significantly higher in late-season controls than in early-season reproductive controls ( $N = 60$ ,  $\chi^2 = 5.55$ ,  $DF = 1$ ,  $P = 0.0200$ ).

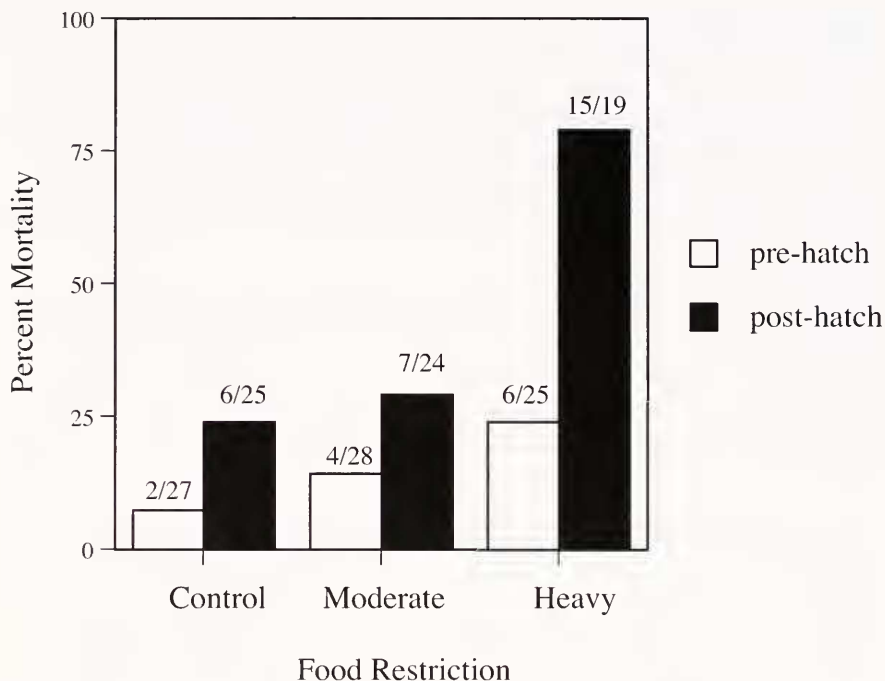


Figure 1. Percent pre-hatching (clear bars) and post-hatching (black bars) mortality in unrestricted (control) and moderately/heavily food-restricted reproductive females. Numbers over bars are frequency/total for each group.

## METHODS II: PRE-REPRODUCTIVE FOOD RESTRICTION

The previous experiments were characterized by two patterns. First, heavy food restriction during reproduction was associated with high maternal mortality following the appearance of offspring. Second, females that presumably reproduced at least once suffered high mortality even when food was plentiful. It seems unrealistic, however, that food resources would suddenly disappear under natural conditions. Hence a more biologically meaningful experiment would involve food restriction prior to the onset of reproduction.

*A. vulgare* were again hand-collected in early May of 2002 in Essex County, New Jersey, USA. Pre-experimental treatment of animals was identical to that of the previous year with the following exceptions. First, only two experimental groups were constructed: control females with unlimited access to carrots throughout the experiment and heavily food-restricted females treated with a feeding cycle of two days access to food followed by four days without food. Second, food-restricted females were placed on this feeding cycle immediately after capture. Hence these females experienced diminished food resources prior to the first reproductive episode of the season.

We collected 106 females and divided them into the two treatment groups by matched pairs according to estimated body size. Upon evidence of egg bearing (determined by visual inspection), females were isolated into individual *Drosophila* culture vials. Females were examined daily for the continued presence of eggs, the occurrence of spontaneous termination of care, or the death of the subject. We also estimated fecundity as the number of young to emerge from the marsupium in successful females. Observations ended 43 days after the experiment began, when all females that reproduced had either successfully hatched young or died prior to hatching.

## RESULTS II

### PRE-REPRODUCTIVE FOOD RESTRICTION

There were no differences in the occurrence of oogenesis between treatment groups. Of the 53 control females, 28 became gravid, whereas 21 of 53 food-restricted females became gravid ( $N = 106$ ,  $\chi^2 = 1.86$ ,  $DF = 1$ ,  $P = 0.1727$ ). There were also no differences in pre-hatching or post-hatching maternal mortality. Seven control and three restricted females died without hatching young ( $N = 49$ ,  $\chi^2 = 0.85$ ,  $DF = 1$ ,  $P = 0.3571$ ) and nine control and seven restricted females died after the young hatched ( $N = 39$ ,  $\chi^2 = 0.06$ ,  $DF = 1$ ,  $P = 0.8017$ ). There were also no differences observed in fecundity, with control females producing an average of 20.19 hatched offspring ( $SE = 2.107$ ) and restricted females producing an average of 20.28 ( $SE = 2.181$ ) (T-test,  $N = 49$ ,  $T = 0.03$ ,  $P = 0.9773$ ).

There was a statistical trend, however, for control females to initiate reproduction earlier than food-restricted females (Fig. 2). Control females became visibly gravid after an average of 9.79 days ( $SE = 1.713$ ), while this occurred in food-restricted females after an average of 14.81 days ( $SE = 2.374$ ) (T-test,  $N = 49$ ,  $T = 1.76$ ,  $P = 0.0846$ ).

## DISCUSSION

Perhaps the most interesting outcome of this study was that while females appeared capable of terminating post-embryonic maternal investment, they only did so with low frequency and without obvious pattern. In several insect taxa, spontaneous termination of care is an active reproductive strategy (Coleoptera: Silphidae, Scott and Gladstein 1993; Heteroptera: Cydnidae, Kight 1997; Heteroptera: Belostomatidae, Kight et al. 2000). In the present study, however, only a few cases of reversal from gravid to non-gravid condition were observed in all treatment groups, regardless of food availability. This might be expected if most females were nearing senescence. However, this seems unlikely because terrestrial isopods survive and reproduce across multiple years and samples most likely contained females from a range of ages and reproductive histories. We must therefore conclude that either active termination of care has not evolved as a reproductive strategy in *A. vulgare*, or that diminished food resources are insufficient



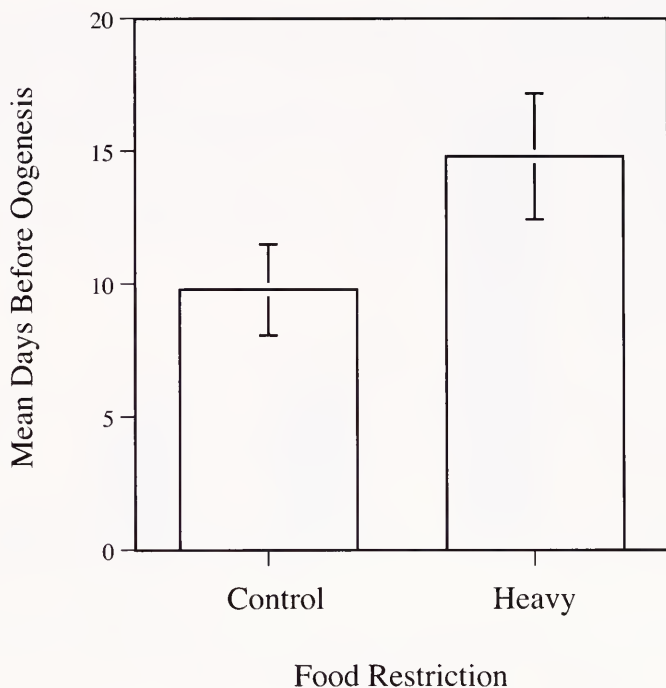


Figure 2. Average time (days) between capture and oogenesis in unrestricted (control) and heavily food-restricted reproductive females. Error bars represent standard error on the mean.

to elicit the response. The latter might be expected if food resources are rarely limited under natural conditions, but we have no data to address this hypothesis.

The most obvious effect of food restriction was seen in the relatively high post-reproductive mortality of females that were heavily food-restricted after oogenesis. Rather than terminate investment in the face of diminished resources, these females appeared to expend reserves that might otherwise have been used for post-reproductive maintenance and survival.

It is interesting that late-season females exhibited high mortality rates regardless of food availability. These females had presumably reproduced at least once prior to capture, and perhaps multiple times. Souty-Grosset et al. (1988) observed three parturial molts in some populations of *A. vulgare*. Late-season females may therefore have had low reserves for somatic maintenance and survival. This hypothesis is supported by the low overall mortality of early-season reproductive controls relative to late-season controls. It should be noted, however, that this difference is also consistent with age-related increases in mortality. These are not mutually exclusive hypotheses and could potentially have an additive effect on mortality.

In contrast, when food restriction was applied prior to oogenesis the overall mortality of food-restricted females diminished to a level not different from reproductive controls. This could be an adaptive outcome of the delay with which food-restricted females began reproduction. For example, females facing diminished resources may have physiologically adapted or behaviorally compensated prior to oogenesis, either by eating more food when it was available, producing smaller eggs, etc. Although our data do not address these hypotheses, we may reasonably rule out an adjustment in fecundity, which was not different between treatment groups at the time of offspring dispersal.

The tendency of food-restricted females to delay oogenesis, which we discovered only after the experiment ended, exposed an experimental design difficulty for the second year of the study. Because restricted females delayed reproduction, the portion of the observation period in which they were classified as post-reproductive was shorter than that of control females. This may have inflated the measure of post-reproductive mortality in control females. The alternative would have been to observe all females for some standard length of time after the hatching of young, but this could have inflated the measure of post-reproductive mortality in food-restricted females because they would have been older. Hence we must caution that our data provide a very conservative estimate of post-reproductive mortality in females that are food restricted prior to oogenesis.

We may reasonably conclude that brood bearing in female *A. vulgare* is energetically expensive. This expense appears to be met through increased post-reproductive mortality when food resources are limited after oogenesis or in delayed reproduction when food is scarce prior to oogenesis. Although active termination of care does not appear to be an important part of reproductive behavior in this species, reduced mortality suggests that delaying reproduction may be an effective strategy for maximizing reproductive success when food resources are limited.

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